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Ontogenetic Changes in Enzyme Distribution and Midgut Function in Developmental Stages of *Penaeus setiferus* (Crustacea, Decapoda, Penaeidae)

DONALD L. LOVETT* AND DARRYL L. FELDER

Department of Biology and Center for Crustacean Research, University of Southwestern Louisiana, Lafayette, Louisiana 70504

Abstract. Ultrastructure and histochemical distribution of enzymes were examined in the midgut of larval and postlarval stages of *Penaeus setiferus*. Acid phosphatase and esterase activities were present in all gut tissues at all stages. Protease activity was present in the anterior and lateral midgut caeca, as well as in the anterior portion of the midgut trunk (MGT) of larvae and early postlarvae (PL_1–PL_4). Amylase activity could not be detected histochemically in larvae or early postlarvae, even though it was detected in assays of whole-animal homogenates. In later postlarvae, both protease and amylase activities were present in the hepatopancreas and anterior MGT, but were absent from the anterior midgut diverticulum.

In larvae, alkaline phosphatase activity is present throughout the midgut, suggesting that absorption is widespread. In juveniles, activity is restricted to the hepatopancreas and regions of the MGT within the cephalothorax. The abdominal MGT (or "intestine") is no longer absorptive by the time the hepatopancreas has attained its adult form. Although epithelial cells of the MGT synthesize protein and produce electron-dense secretory vesicles, they are substantially different in ultrastructure from those cells in the hepatopancreas responsible for digestive enzyme synthesis and secretion.

Epithelial cells of the larval anterior and lateral midgut caeca are structurally and functionally similar to cells of the postlarval hepatopancreas. However, the lateral midgut caeca retain these features as they transform into the hepatopancreas, while the anterior midgut caeca lose these functions as they degenerate into the anterior diverticulum and change in ultrastructure during early postlarval development. The anterior and posterior midgut diverticula of postlarvae are similar ultrastructurally even though they differ in ontogenetic history.

Introduction

In crustaceans, the foregut and hindgut are chitin-lined, while the intervening midgut is uncuticularized. The midgut is thus the region in which cells are in contact with the lumen of the alimentary canal. It comprises a tubular portion, which we call the "midgut trunk" (MGT)[1], and the various outpocketings (diverticula and caeca) of this MGT (Lovett and Felder, 1989).

In adult penaeids, such as *Penaeus setiferus* (Linnaeus, 1767), outpocketings of the MGT include the complex hepatopancreas (= digestive caeca or midgut gland, by some authors), a single anterior midgut diverticulum at the junction of the foregut with the MGT, and the posterior midgut diverticulum at the junction of the MGT with the hindgut (Dall, 1967a). These adult structures arise, during ontogeny, by the progressive transformation of several larval structures: a pair of anterior caeca located at the foregut-MGT junction, and a pair of lateral caeca that arise slightly posteriad to the foregut-MGT junction. During late larval and early postlarval development, the two anterior caeca decrease in relative and actual size, fuse medially, and begin to form the single ante-

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* Present address: Department of Biology, Lake Forest College, Lake Forest, Illinois 60045.

[1] The term "intestine" has usually been used to refer to this tubular region of the midgut. But we and others consider this to be a misapplication of vertebrate terminology and a practice to be discouraged (Dall and Moriarty, 1983; Lovett and Felder, 1989).

rior midgut diverticulum of the adult. At about the same time, the adult hepatopancreas is derived by ramification of the larval lateral midgut caeca. Finally, and in contrast, the adult posterior midgut diverticulum first makes its appearance during the third week of postlarval development. The ontogeny of the penaeid gut is described in detail elsewhere (Lovett and Felder, 1989, 1990a).

The ultrastructure and function of the crustacean midgut is only partly known, and most work has been done with adult specimens only. The adult hepatopancreas has been well-studied in *Penaeus* (Al-Mohanna *et al.*, 1985a, b; Vogt, 1985; Vogt *et al.*, 1985, 1986; Al-Mohanna and Nott, 1986; Caceci *et al.*, 1988) and in other decapod crustaceans (see Gibson and Barker, 1979; Dall and Moriarty, 1983), but relatively little attention has been given to other regions of the midgut.

Functions previously attributed to the crustacean MGT include: (1) absorption of nutrients from digested food (Yonge, 1924; Reddy, 1937; Speck and Urich, 1970; Talbot *et al.*, 1972; Ahearn, 1974; Quaglia *et al.*, 1976; Ahearn and Maginniss, 1977; Barker and Gibson, 1977, 1978; Brick and Ahearn, 1978; Gemmel, 1979); (2) absorption of ions and control of net water flux between the midgut lumen and the hemolymph (Yonge, 1924; Croghan, 1958; Green *et al.*, 1959; Gifford, 1962; Dall, 1965, 1967b; Geddes, 1975; Malley, 1977; Ahearn *et al.*, 1978; Mykles and Ahearn, 1978; Mykles, 1979, 1980, 1981; Ahearn, 1980, 1982, 1984; Wyban *et al.*, 1980); (3) excretion of ions (Green *et al.*, 1959; Gifford, 1962; Dall, 1967b, 1970); and (4) secretion of the peritrophic membrane (Georgi, 1969; Mykles, 1979; Johnson, 1980; Dall and Moriarty, 1983). Some of these functions, in particular the absorption of nutrients, have been assigned on the basis of an assumed analogy between the MGT and the vertebrate intestine.

The functions of the anterior and posterior midgut caeca and diverticula remain obscure; yet some proposals have been made: (1) both may increase surface area of the midgut for absorption of either water at ecdysis (Holliday *et al.*, 1980) or nutrients during digestion (Yonge, 1924; Reddy, 1937); (2) both may secrete digestive enzymes (Holliday *et al.*, 1980); (3) both may function in excretion (Dall, 1967b); (4) both may function in ion and water balance (Young, 1959; Heeg and Cannone, 1966; Dall, 1967b; Mykles, 1977, 1979); (5) both may secrete the peritrophic membrane (Pugh, 1962; Dall, 1967a; Georgi, 1969; Mykles, 1979); (6) both serve as sources of replacement cells (sites of cell regeneration) for the hepatopancreas and MGT (Davis and Burnett, 1964; Johnson, 1980); (7) the anterior diverticulum may accommodate volume change during contraction of the foregut (Powell, 1974); and (8) the anterior diverticulum may contribute essential components of digestive fluid that function in activation of proteolytic enzymes or pH change (Dall and Moriarty, 1983).

In most decapod crustaceans, the adult form of the gut appears immediately following metamorphosis (Felder *et al.*, 1985). However, in *Penaeus setiferus*, transformation of the gut to the adult form is protracted, taking place over several weeks after metamorphosis (Lovett and Felder, 1989, 1990a). Thus, this shrimp has enabled us to correlate the development of digestive function with that of structure. Toward that end, we have investigated the ontogenetic changes in the distribution of digestive enzymes in *Penaeus setiferus* and have compared those changes with simultaneous ontogenetic transformations in midgut ultrastructure, gross morphology, and movement.

Materials and Methods

Specimens examined

Larvae of *Penaeus setiferus* were reared in the laboratory with natural seawater (for details, see Lovett and Felder, 1990b) on a diet of algae (*Isochrysis* sp., *Chaetocerus gracilis*, and *Tetraselmis chuii*) and 24-h *Artemia* nauplii, by the method of McVey and Fox (1983). Beginning with PL_5 (the fifth day of postmetamorphic life), the diet consisted entirely of *Artemia* nauplii. Larval stages (protozoea and mysis) were identified in accord with descriptions by McVey and Fox (1983). Postlarval (PL_n) stages are identified by postmetamorphic age (where n = days beyond metamorphosis), as is the practice in culture of penaeid shrimp. "Juveniles" examined were at postlarval stage PL_{140}.

Histochemical localization of enzymes

Sample preparation. Because diel rhythmicity in enzyme activity has been reported for adults of *Penaeus* (Van Wormhoudt *et al.*, 1972; Cuzon *et al.*, 1982), all specimens were collected in mid-morning when peak enzyme activities reportedly occur. Food was available continually and the guts of all specimens were filled with food. Specimens were embedded in Tissue-Tek O.C.T. Compound® (Miles Scientific, Naperville, Illinois), quench frozen in liquid nitrogen, and stored until use at −70°C in an ultracold freezer. Serial sections were cut at 8 μm thickness with a Miles Cryostat II. All slides used in this study were coated with a chrome alum-gelatin subbing solution (Pappas, 1971). Sections were placed on cold (−25°C) slides (but see amylase and protease tests below) and then melted by placing a thumb on the underside of the slide. Slides were allowed to dry at room temperature before incubation. Control sections for alkaline phosphatase, acid phosphatase, and esterase tests were immersed in 90°C water for 5 min before incuba-

tion. After incubation, sections were counterstained with Mayer's haemalum; color was developed by dipping sections in Scott's solution (2% $MgSO_4$ with 0.2% $NaHCO_3$). Sections were mounted in glycerol gel.

Reconstruction of serial sections. Distribution of sites of enzyme activity within the midgut was determined by reconstruction of serial sections. To determine the abdominal segment within which sites of activity occurred, the product of total number of sections multiplied by 8 μm was compared with average total length and length of each abdominal segment for the respective developmental stage.

Non-specific esterase. Sections were incubated for 30 min at room temperature in a 0.01% solution of Naphthol AS-LC acetate by a method adapted from Burstone (1962): substrate solution was made by dissolving 5.0 mg Naphthol AS-LC acetate in 1.0 ml N,N-dimethylformamide. After the substrate had dissolved, 10 ml of ethylene glycol monomethyl ether was added, followed by 10 ml of 0.2 *M* Tris (hydroxymethyl) aminomethane hydrochloride buffer at pH 7.1. Immediately before incubation, 40 mg of Fast Garnet GBC dissolved in 29 ml of distilled water were filtered into the substrate solution. Cholinesterase activity was inhibited by adding $10^{-5}M$ eserine to the final substrate solution. Sites of esterase activity were indicated by deep violet precipitate.

While esterase activity was detected successfully with Naphthol AS-LC acetate as the substrate, neither esterase nor lipase activity was detected when several other substrates were used. When other substrates were used to incubate frozen sections and paraffin sections of fresh, formalin-fixed, acetone-fixed, and freeze dried specimens, the following results were obtained: The 5-bromoindoxyl acetate substrate by the method of either Barrnett and Seligman (1951) or Holt and Withers (1952) yielded a highly diffuse, faint blue precipitate that was unsuitable for study. Incubation of sections with Tween 20, 40, 60, or 80 substrates by the method of Gomori (1945, 1949) and with Tween 85 by the method of Bokdawala and George (1964), followed by demonstration of calcium soaps with either yellow ammonium sulfide or Alizarin Red S, yielded a diffuse precipitate that could not be differentiated from that obtained in control sections heated at 90°C for 5 min prior to incubation.

Alkaline phosphatase. Sections were incubated for 25 min at room temperature in a 0.02% solution of Naphthol AS-MX phosphate free acid with 0.06% Fast Red Violet LB salt at pH 8.5 by the method of Burstone (1962). Sites of alkaline phosphatase activity were indicated by magenta precipitate.

Acid phosphatase. After sections had dried on the slide, they were fixed in 4°C 10% neutral formalin for 30 s and washed for 3 min to localize the reaction. Sections were incubated for 30 min at 37°C in a 0.02% solution of Naphthol AS-BI phosphate with 0.06% Fast Red Violet LB salt at pH 5.2 using Burstone's (1958, 1962) method. Sites of acid phosphatase activity were indicated by a magenta precipitate.

Amylase. By a technique modified from that of Tremblay and Charest (1968), slides coated with chrome alum-gelatin subbing were dipped in a solution of 4% purified potato starch in 20 m*M* phosphate buffer pH 6.9 with 10 m*M* NaCl and dried at room temperature. The solution had been boiled, filtered, and degassed under vacuum prior to use. Frozen sections were placed on the starch substrate film of slides that were prechilled to −25°C. Sections were melted and air-dried at room temperature. Slides were then incubated at 37°C for 1–2 h in covered petri dishes lined with water-soaked filter paper. Slides were thereafter air-dried, immersed in a solution of 5:1:5 methanol:acetic acid:distilled water for 15 min, treated with Periodic Acid-Schiff's (PAS) by the method of McManus (1948), and air-dried again. Sites of amylase activity were indicated by clear areas where the starch film (now stained magenta) had been digested away.

Protease. Sections were incubated on the gelatin emulsion of Kodachrome-25™ color transparency film that had been previously exposed to daylight and developed commercially. By a technique adapted from Fratello (1968), sections were placed on emulsion prechilled to −25°C. Sections were then melted, air-dried at room temperature, incubated at 37°C for 1–2 h in petri dishes lined with water-soaked filter paper, and then dried again at room temperature. No buffer was added to control pH. Sites of protease activity were indicated by light blue-green or white areas where the darkly colored emulsion had been digested away.

Activity of specific proteolytic enzymes were not detected successfully. Sections were incubated with N-(α-benzoyl-DL-arginine-β-naphthylamide) hydrochloride in the method of Glenner and Cohen (1960) to detect trypsin-like activity and with both L-leucyl-β-naphthylamide by the methods of Burstone and Folk (1956) and Loizzi and Peterson (1971) and L-leucyl-4-methoxy-naphthylamide hydrochloride by the method of Nachlas *et al.* (1960) to detect arylamidase (aminopeptidase) activity. Regardless of the method used for fixation and embedment, all preparations yielded results not different from controls.

Transmission electron microscopy

Specimens were fixed in cold (4°C) 4% glutaraldehyde solution buffered to pH 7.2 with 0.2 *M* phosphate buffer and postfixed with 2% buffered osmium tetroxide. Specimens were dehydrated in acetone, infiltrated by centrifugation at 2500 rpm (after Millonig, 1976), and embedded in Spurr's low viscosity resin (obtained from Polysci-

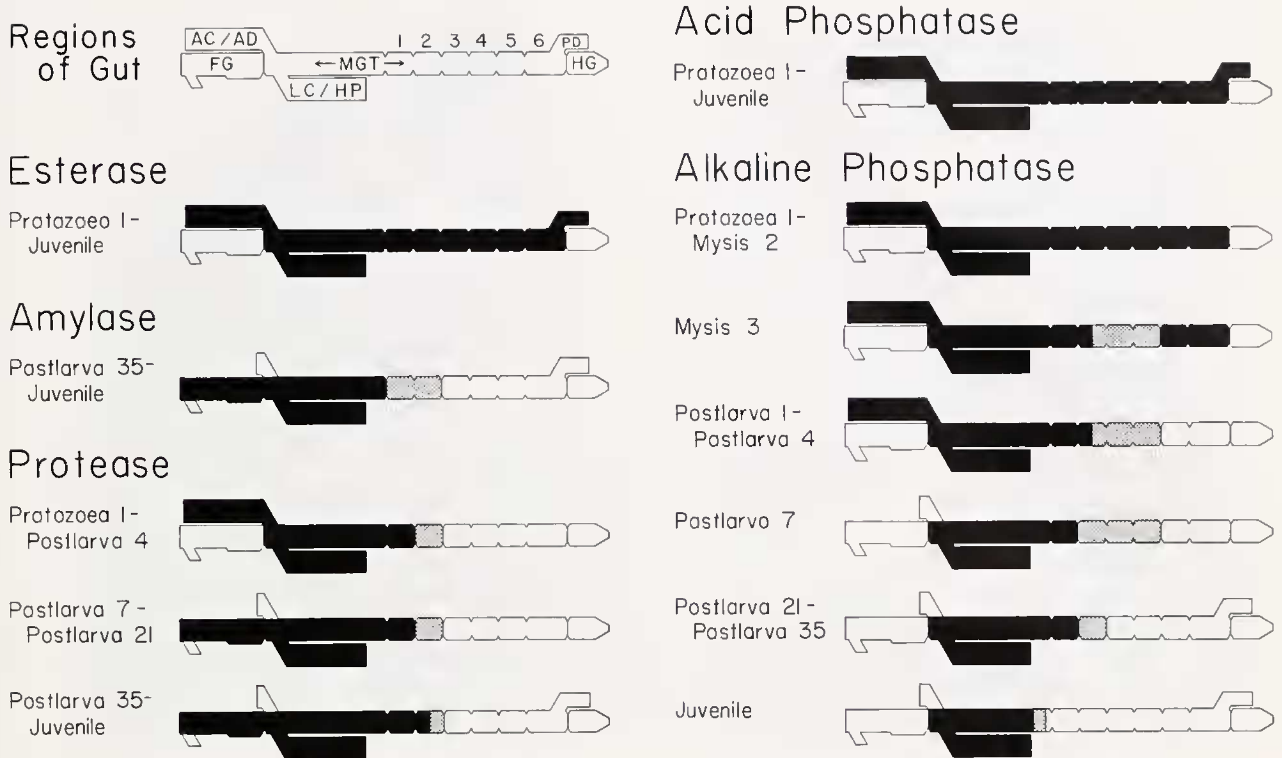

Figure 1. Diagrammatic representation of a lateral view of the gut in *Penaeus setiferus* illustrating distribution of enzymes during development. AC, anterior midgut caeca; AD, anterior midgut diverticulum; FG, foregut; HG, hindgut; HP, hepatopancreas; LC, lateral midgut caeca; MGT, midgut trunk (= "intestine"); PD, posterior midgut diverticulum. Abdominal segments 1–6 are numbered. Label to left indicates developmental stages included for each diagram. Solid black areas indicate regions of gut where presence of enzyme was detected in all specimens. Stippled areas indicate regions where enzyme was detected in some, but not all specimens.

ences, Inc., Warrington, Pennsylvania). Material was sectioned both with glass and diamond knives on a Sorval MT-5000. Ultrathin sections of 80–90 nm were stained with methanolic uranyl acetate and lead citrate and examined at 75 kV with an Hitachi H-600 transmission electron microscope.

Results

Ontogenetic change in enzyme distribution

Non-specific esterase. Esterase activity was found in all regions of the midgut in all stages examined (Figs. 1, 2b).

Amylase. No amylase activity was detected histochemically in developmental stages before PL_{35}. In late postlarval stages activity was found in the hepatopancreas, anterior portion of the MGT, and the lumen of the foregut (Fig. 1). Because of the nature of the test it could not be determined with certainty whether activity in the MGT was restricted to either the extraperitrophic or endoperitrophic lumen. No activity was found in the anterior diverticulum or in any areas of the gut posteriad to abdominal segment 2.

Protease. In all larval and postlarval stages, protease activity was found in the hepatopancreas and in the anterior region of the MGT (Fig. 1). Resolution was inadequate to differentiate intracellular activity from luminal activity. Furthermore, it could not be determined whether activity was restricted to either the extraperitrophic or the endoperitrophic lumen of the MGT. Activity was never found posteriad to abdominal segment 2. In larval and early postlarval stages (PL_1–PL_4), protease activity was found in the anterior midgut caeca, but not in the foregut. When the anterior caeca had degenerated into the anterior diverticulum, activity no longer was found in this caecal extension of the midgut, but activity then was found in the lumen of the foregut.

Acid phosphatase. Acid phosphatase activity was found in all regions of the midgut in all developmental stages (Figs. 1, 2d).

Alkaline phosphatase. Alkaline phosphatase activity was detected in the hepatopancreas and the anterior region of the MGT in all developmental stages (Figs. 1, 3d). However, distribution of alkaline phosphatase for the remaining regions of the midgut becomes limited during

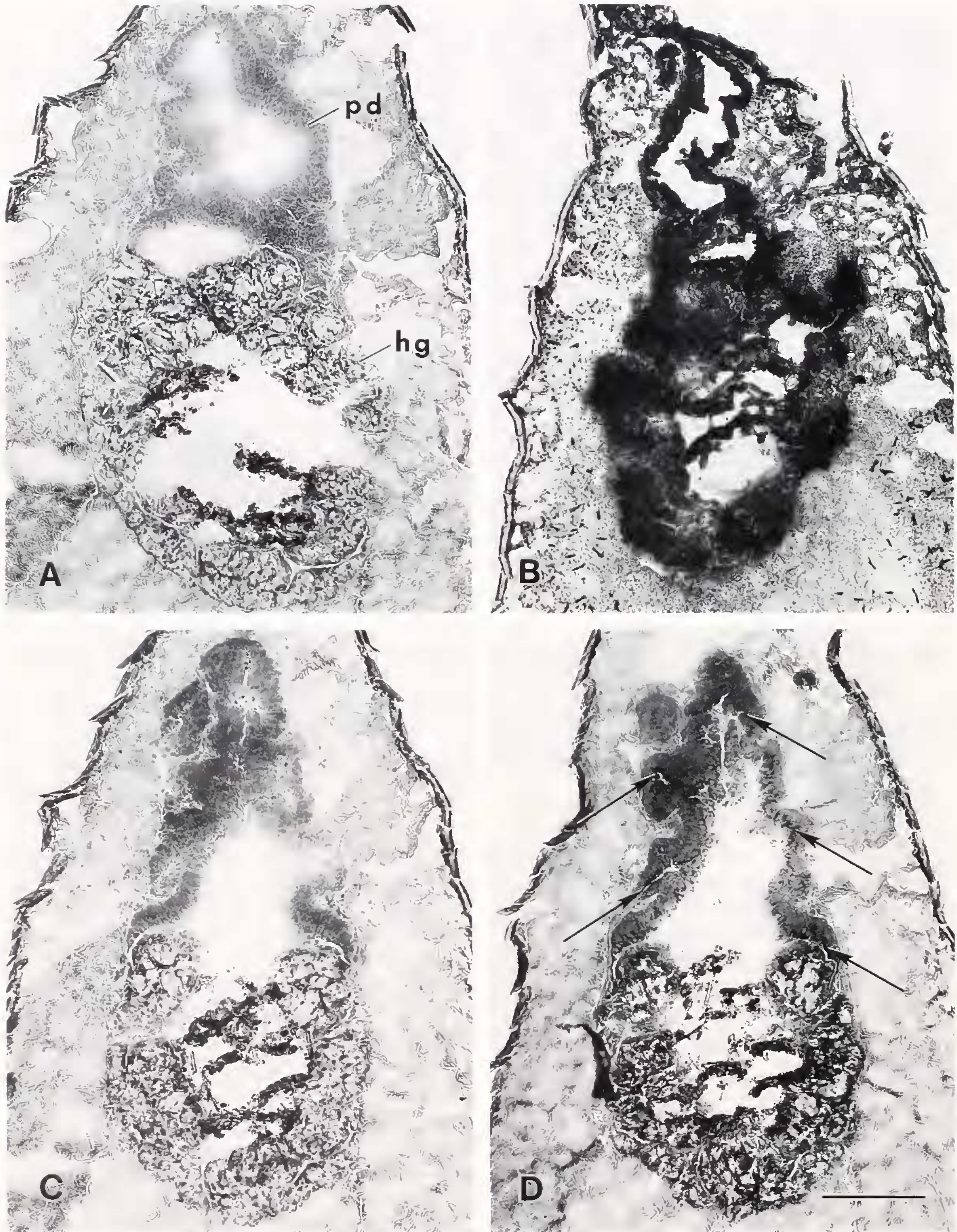

Figure 2. Histochemical localization of enzymes in fresh frozen transverse sections of posterior midgut diverticulum and hindgut in *Penaeus setiferus* juveniles (PL_{140}). A, control section. B, section incubated with Naphthol AS-LC acetate as substrate to indicate esterase activity. C, section incubated with Naphthol AS-BI as substrate to indicate alkaline phosphatase activity (no precipitate present). D, section incubated with Naphthol AS-MX phosphate as substrate to indicate acid phosphatase activity [in diverticulum, precipitate present (arrows) along apical surfaces of cells]. (hg, hindgut; pd, posterior midgut diverticulum). Scale bar indicates 150 μm for all figures.

development. For all larval and early postlarval stages, activity was found in the anterior midgut caeca (Fig. 3b). In PL_1 and PL_4, alkaline phosphatase activity was always more intense in the anterior caeca than in any other region of the midgut. However, no activity was detected in the anterior diverticulum of subsequent postlarval stages. Activity was found along the entire length of the MGT in larval stages Protozoea 1 through Mysis 2 (Fig. 3d, f). However, all specimens of Mysis 3 larvae had a short region in the MGT between the middle of abdominal segment 2 and the end of abdominal segment 4, in which no activity was found (Fig. 1); the exact location of this region varied from specimen to specimen. In PL_1 and PL_4, no activity could be demonstrated posteriad of abdominal segment 2 in some specimens, while in all specimens of these stages no activity was detected posteriad of abdominal segment 4. During development, the posterior limit of alkaline phosphatase activity progressed anteriad until, in the juvenile (PL_{140}), no activity was found in any portion of the abdominal MGT (Fig. 3h). Alkaline phosphatase activity was never demonstrated in the posterior midgut diverticulum (Fig. 2c).

Ontogenetic change in ultrastructure

In larval and early postlarval stages, the ultrastructure of cells of the anterior midgut caeca resembled that of cells of the lateral midgut caeca (Fig. 4a, b). However, the degeneration of the anterior caeca into the anterior diverticulum was accompanied by considerable change in the ultrastructure of the epithelial cells. The cells became elongate and no longer contained large vacuoles. In some cells, particularly in those ventral to the lumen of the diverticulum, the cytoplasm and all recognizable organelles became electron dense (Figs. 4c, 5a). Because adjacent cells varied in electron density (Fig. 5a, b), this density was not attributable to thick sections or overstaining.

Cells of the anterior diverticulum bore apical microvilli with a glycocalyx. Golgi bodies had swollen cisternae and produced secretory granules similar to those produced by the MGT. The lateral cell membranes were distinctly undulatory in nature. Where the epithelium of the anterior diverticulum tapered into the MGT, a mosaic of cell types was present (Fig. 5b). Epithelial cells of the posterior midgut diverticula (Fig. 5c) were similar in ultrastructure to those of the anterior diverticulum.

In all developmental stages of *Penaeus setiferus*, epithelial cells of the MGT had apical microvilli with a distinct glycocalyx (Fig. 6). Active Golgi produced electron-dense secretory vesicles, which accumulated in the apical cytoplasm. Cisternae of the smooth endoplasmic reticulum were often distended. Rough endoplasmic reticulum usually was dense and its membranes were arranged in parallel rows. Intracellular lipid droplets were found occasionally in cells of the MGT, within both the cephalothorax and first abdominal segment.

Discussion

In the early postlarval stages of *Penaeus setiferus*, both the anterior and lateral midgut caeca secrete digestive enzymes, and the entire midgut is absorptive. As the two anterior caeca degenerate into the single anterior diverticulum, there is tremendous change in both function and ultrastructure: the capacity for both secretion of digestive enzymes and absorption is lost; the epithelium changes from being ultrastructurally similar to that of the adult hepatopancreas to being ultrastructurally similar to that of the posterior midgut diverticulum, even though the latter has an independent ontogenetic origin. As the hepatopancreas differentiates and increases allometrically in size, the MGT loses its absorptive capacity. Contrary to some reports, the abdominal MGT (or "intestine") does not absorb digested food substrates once the gut has attained the adult form.

Enzyme distribution

With a few exceptions (notably Holliday *et al.*, 1980), the histochemical distribution of enzymes observed in *Penaeus setiferus* is consistent with that reported for other species of decapod crustaceans (Travis, 1955, 1957; Miyawaki *et al.*, 1961; Davis and Burnett, 1964; Loizzi, 1966; Van Herp, 1970; Loizzi and Peterson, 1971; Momin and Rangneker, 1974, 1975; Barker and Gibson, 1977, 1978). Although arylamidase activity was reported in hepatopancreatic cells of *Scylla* (Barker and Gibson, 1978), neither arylamidase nor aminopeptidase activity has been demonstrated unequivocally in histochemical studies of any other species of decapod. In tissue homogenates of *P. setiferus*, we measured significant amylase activity for all developmental stages, but activity remained low until late in postlarval development (Lovett and Felder, 1990b). Lack of histochemical evidence for amylase activity in larval and early postlarval stages of *P. setiferus* suggests that concentrations were below the limits of detection for the technique used.

Lateral midgut caeca and hepatopancreas

Both acid phosphatase and esterase activities within the hepatopancreas of decapods have been associated with the synthesis and secretion of digestive enzymes by this tissue, whereas alkaline phosphatase activity in the hepatopancreas has been associated with transmembrane transport of metabolites (Momin and Rangneker, 1974; Barker and Gibson, 1977, 1978; Lane, 1984). Although the exact function of alkaline phosphatase in ab-

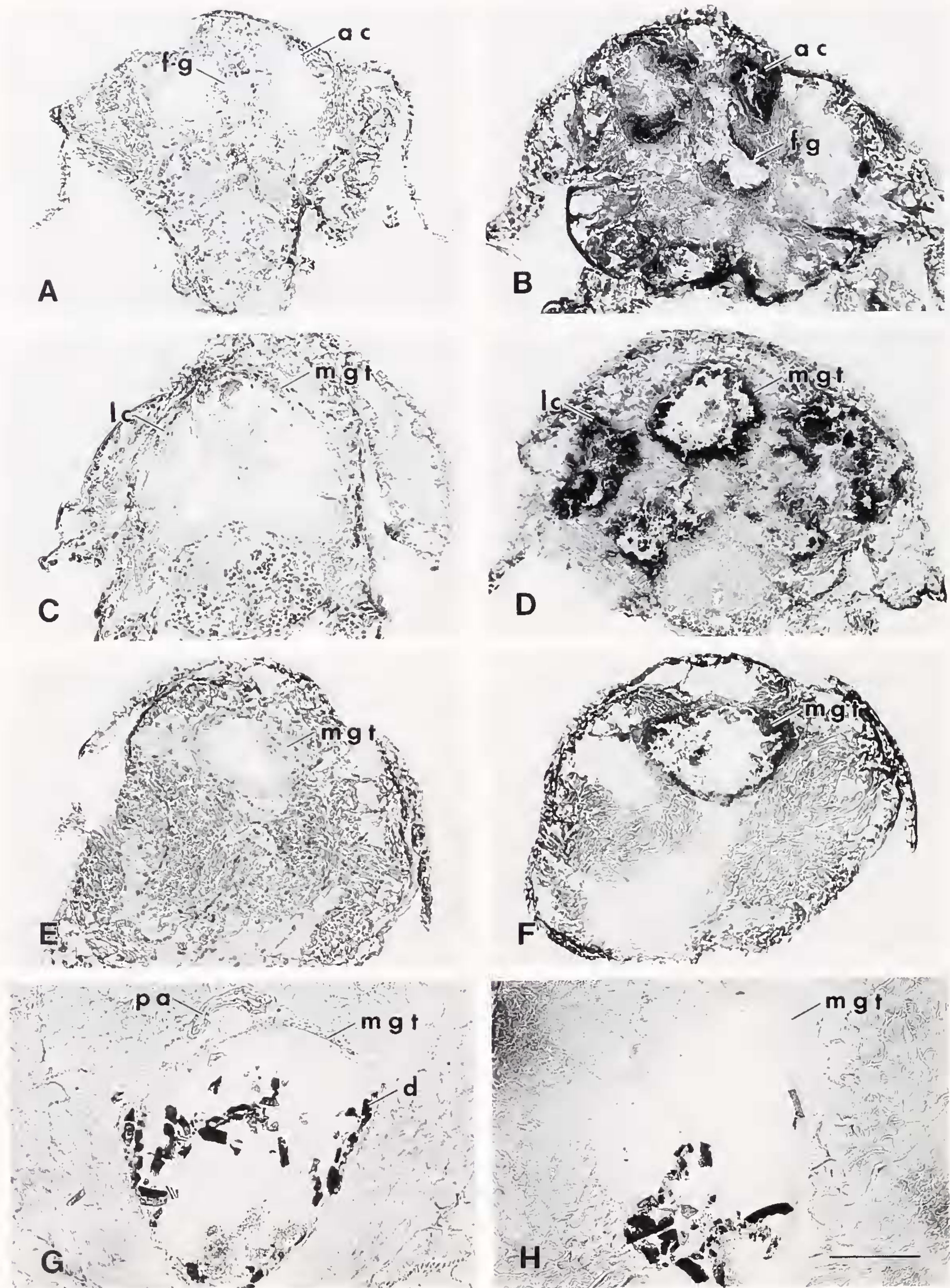

Figure 3. Histochemical localization of alkaline phosphatase activity in fresh frozen sections of *Penaeus setiferus.* A, C, E, G, control sections. B, D, F, H, sections incubated with Naphthol AS-BI phosphate as substrate. A, B, transverse section through foregut and anterior midgut caeca of larval stage Mysis 2. C, D, transverse section through lateral midgut caeca and midgut trunk of Mysis 2. E, F, transverse section through abdominal segment 2 of Mysis 2. G, H, transverse section through abdominal segment 2 of juvenile (PL_{140}). (ac, anterior midgut caecum; fg, foregut; d, debris in lumen; lc, lateral midgut caecum; mgt, midgut trunk; pa, posterior artery). Scale bar indicates 100 μm for A–F and 125 μm for G and H.

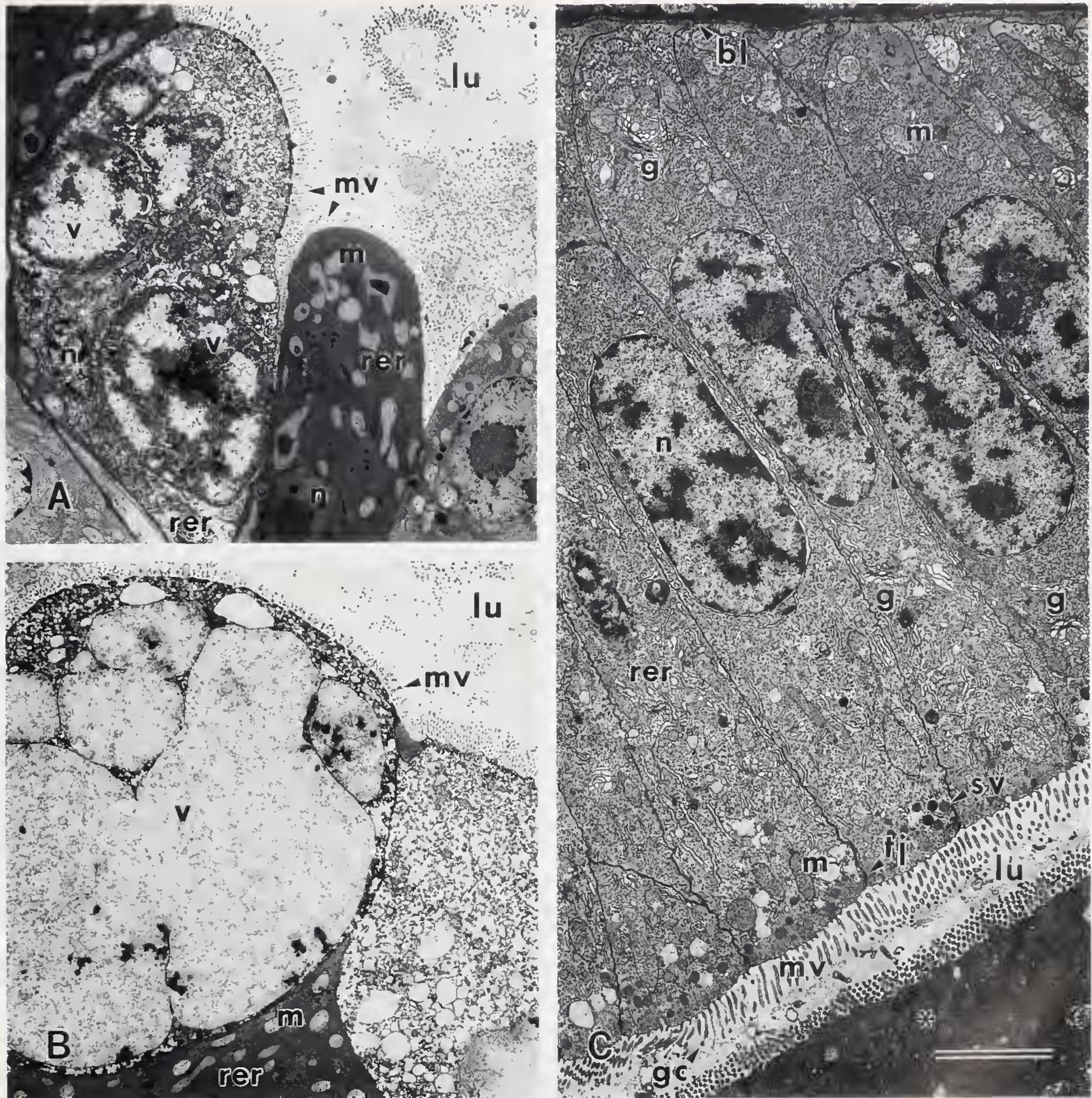

Figure 4. Epithelia of larval midgut caeca and postlarval anterior midgut diverticulum in *Penaeus setiferus*. A, lateral midgut caecum. B, anterior midgut caecum. C, anterior midgut diverticulum. (bl, basal lamina; g, Golgi bodies; gc, glycocalyx; lu, lumen; m, mitochondrion; mv, microvilli; n, nucleus; rer, rough endoplasmic reticulum; sv, secretory vesicle; tj, tight junction; v, vacuole; asterisk indicates electron dense epithelium ventral to lumen of anterior diverticulum; arrow indicates undulatory lateral membranes). A, B, larval stage Protozoea 3. C, postlarval stage PL_{35}. Scale bar indicates 6.8 μm for A and B and 4 μm for C.

sorption has yet to be demonstrated, tissues in which alkaline phosphatase activity is present are generally thought to function in absorption by active transport (see review by McComb *et al.*, 1979). Localization of amylase and protease activity within the hepatopancreas in the present study is consistent with previous detection of amylase and tryptic activity within B-cells of the hepatopancreas of other decapod species (Malcoste *et al.*, 1983; De-Villez and Fyler, 1986). Localization of alkaline phosphatase in the hepatopancreas of *P. setiferus* in the present study is consistent with the absorption usually attributed to this tissue (Gibson and Barker, 1979; Dall and Moriarty, 1983). Ultrastructure also has been used to infer that the hepatopancreas functions in protein synthesis, secretion, and absorption in *Penaeus* (Al-Mohanna *et al.*, 1985b; Vogt, 1985; Al-Mohanna and Nott,

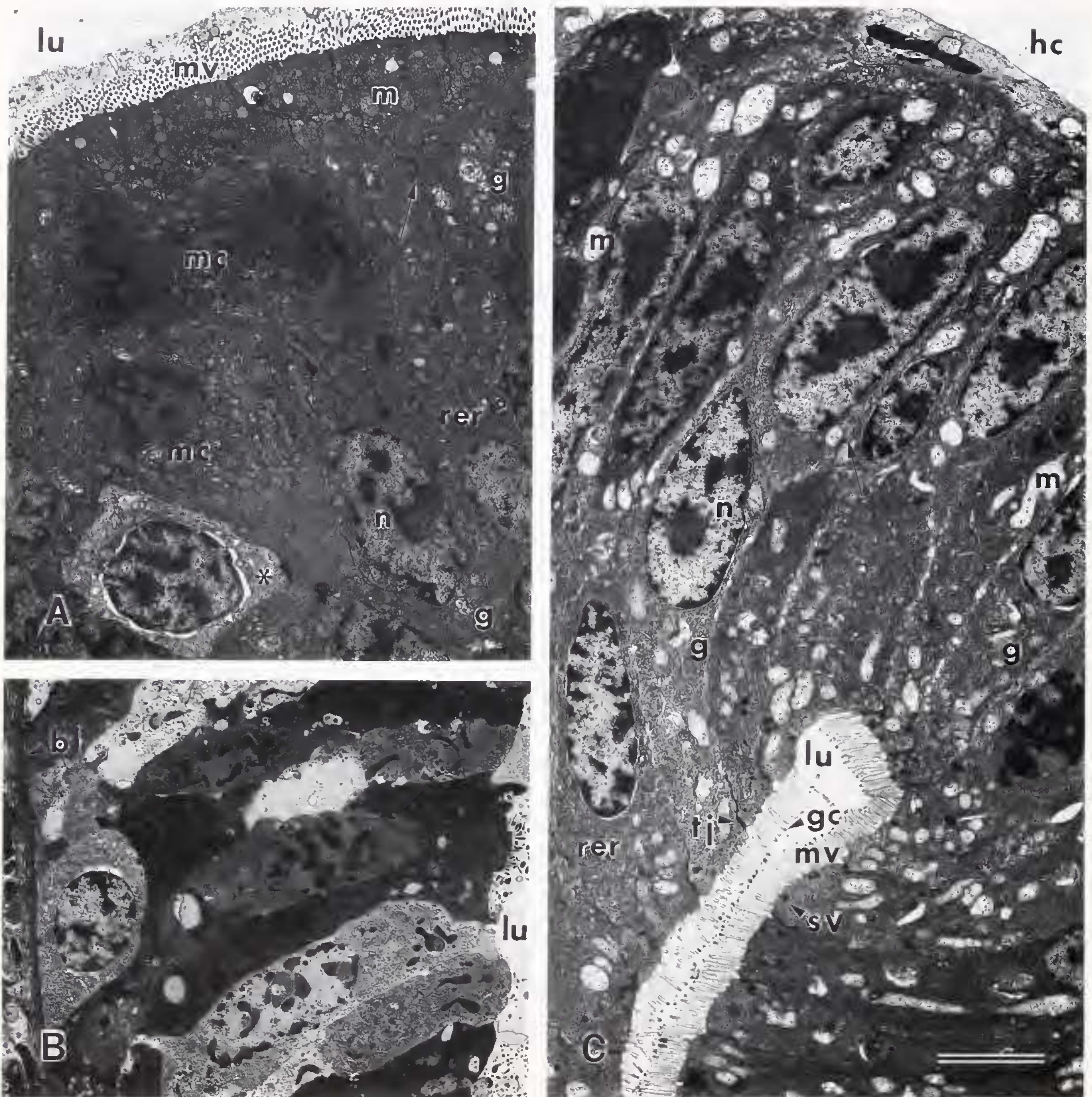

Figure 5. Epithelia of midgut diverticula in *Penaeus setiferus*, postlarval stage PL_{35}. A, anterior midgut diverticulum ventral to lumen (see Fig. 5c); these cells attach to hypodermis of dorsal pyloric valve of foregut; note "normal" cell (asterisk) surrounded by electron-dense cells. B, mosaic of cells where epithelium of anterior diverticulum tapers into midgut trunk. C, posterior diverticulum dorsal to lumen. (bl, basal lamina; g, Golgi bodies; gc, glycocalyx; hc, hemocoel; lu, lumen; m, mitochondrion; mc, cell undergoing mitosis; mv, microvilli; n, nucleus; rer, rough endoplasmic reticulum; sv, secretory vesicle; tj, tight junction; arrows indicate undulatory lateral membranes). Scale bar indicates 4 μm for A and C and 5.8 μm for B.

1986; Caceci *et al.*, 1988), and in other decapod crustaceans (Gibson and Barker, 1979).

In *P. setiferus*, substantial morphological change occurs when the lateral midgut caeca of larvae differentiate into the hepatopancreas during early postlarval development (see Lovett and Felder, 1989). However, except for a decrease in the number and size of lipid droplets within cells during the mysis stages of development, the epithelial cells of the larval lateral caeca and of the mature hepatopancreas are identical in ultrastructure. The amylase, protease, and alkaline phosphatase activities that are evident in the lateral caeca during early development

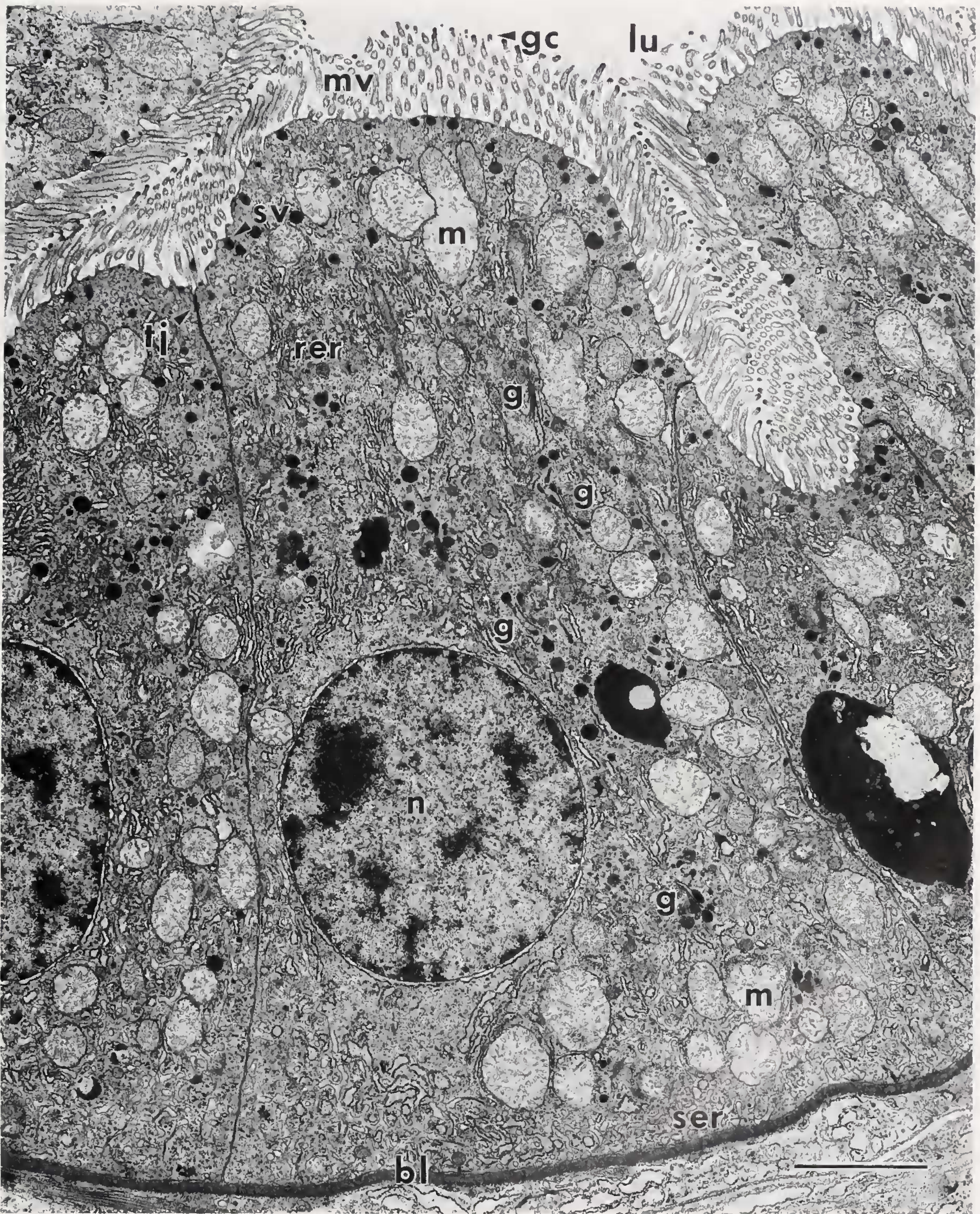

Figure 6. Typical epithelium of midgut trunk in *Penaeus setiferus.* (bl, basal lamina; g, Golgi bodies; gc, glycocalyx; lu, lumen; m, mitochondrion; mv, microvilli; n, nucleus; rer, rough endoplasmic reticulum; ser, smooth endoplasmic reticulum; sv, secretory vesicle; tj, tight junction). Scale bar indicates 3 μm.

are also evident in the mature hepatopancreas. Thus, this region of the gut retains the functions of digestive enzyme synthesis, secretion, and absorption throughout development.

Anterior midgut caeca

In larvae of *P. setiferus*, chyme does not appear to flow from the lateral midgut caeca into the anterior midgut caeca (Lovett and Felder, 1990a). Therefore, protease activity in the anterior midgut caeca most likely represents enzyme that has been secreted by the anterior caeca. Ultrastructural similarity of the anterior midgut caeca with both the larval lateral midgut caeca and the mature hepatopancreas also suggests that the anterior caeca secrete digestive enzymes.

Although chyme does not flow into the anterior caeca from the lateral midgut caeca, it does flow into the anterior caeca from the foregut and the anterior-most portion of the MGT. Absorption in the anterior caeca, as inferred from both alkaline phosphatase activity and ultrastructure, is consistent with the observed movement of chyme into the caeca and secretion of digestive enzymes by the caeca.

Anterior and posterior midgut diverticula

The absence of alkaline phosphatase activity from the anterior diverticulum of postlarvae of *P. setiferus* suggests that this diverticulum is not absorptive, while the absence of amylase or protease activity suggests that it does not secrete digestive enzymes. The apparent postmetamorphic loss of both absorption and the capacity to secrete digestive enzymes in this portion of the midgut is reflected in (1) the complete absence of chyme from the lumen of the anterior diverticulum, and (2) the extensive change in ultrastructure that occurs when the anterior midgut caeca degenerate into the anterior diverticulum. Vacuolated F-cells and B-cells, usually associated with synthesis and secretion of digestive enzymes (Gibson and Barker, 1979), are not present in this portion of the midgut after metamorphosis.

Unlike the anterior midgut diverticulum, which develops from the larval anterior midgut caeca, the posterior midgut diverticulum first differentiates as a distinct structure about three weeks after metamorphosis (Lovett and Felder, 1989, 1990a). Absence of alkaline phosphatase and digestive enzyme activity and absence of chyme from the lumen of the posterior diverticulum suggest that this diverticulum, like the anterior midgut diverticulum, does not function in absorption or digestion. Also, while the anterior and posterior diverticula differ in ontogenetic histories, their epithelia are similar in ultrastructure.

Even though many functions have been proposed for the anterior and posterior diverticula (see Introduction), the precise function of the diverticula remains obscure. As mentioned, neither of these structures appear to function in either the secretion of digestive enzymes or absorption through active transport. However, because the epithelial cells of the diverticula in *P. setiferus* and the mucus-secreting cells in the intestine of mammals both have electron-dense cytoplasm (Ito, 1965), cells of the diverticula may function in secretion of a mucus-like substance. Such a mucous secretion could contribute to the formation of the peritrophic membrane, as proposed by other authors (Pugh, 1962; Dall, 1967a; Georgi, 1969; Mykles, 1979); Holliday *et al.* (1980) dispute this interpretation.

Midgut trunk

Because both absorption in postlarval stages (as inferred from alkaline phosphatase activity) and the presence of digestive enzymes in all developmental stages are restricted to the anterior portion of the MGT in *P. setiferus*, we initially predicted that epithelial cells in the anterior region of the MGT might be differentiated ultrastructurally from those in the posterior MGT. Furthermore, because there is significant ontogenetic change in the distribution of alkaline phosphatase in the MGT, we also predicted that there may be ontogenetic change in ultrastructure of the abdominal MGT during larval and early postlarval development. However, essentially no difference in ultrastructure was found along the length of the MGT and no ontogenetic change in ultrastructure occurred that could be correlated with presence or absence of alkaline phosphatase activity. We also could not distinguish the two types of MGT epithelial cells (light and dark) identified by Talbot *et al.* (1972).

The distribution of acid phosphatase in *P. setiferus* suggests that the entire MGT is involved in active protein synthesis and secretion, and this is consistent with the observed ultrastructure. However, ultrastructural evidence does not necessarily indicate that digestive enzymes present in the anterior MGT are being synthesized and secreted by the MGT. The F-cells and B-cells associated with secretion of digestive enzymes in the hepatopancreas are absent from the MGT. From our *in vivo* observations of flow of chyme within the gut of *P. setiferus*, the observed activity of amylase and protease in the anterior lumen of the MGT likely represents enzymes discharged into the MGT from either the larval midgut caeca or the hepatopancreas. We also observed in all postlarval stages that chyme within the MGT as far posteriad as abdominal segment 2, is regularly "regurgitated" anteriad into the hepatopancreas (Lovett and Felder, 1990a). Moreover, digestive enzymes also occur in the MGT as far posteriad as abdominal segment 2,

where they mix with chyme. These enzymes and the digesting chyme are periodically carried anteriad into the midgut caeca or the hepatopancreas where final digestion and absorption take place.

The presence of apical microvilli, a glycocalyx, tight junctions, and a well-developed basal lamina in epithelial cells along the length of the MGT in adult specimens of *Penaeus* and other decapod crustaceans has led some investigators to conclude that these cells are absorptive (Talbot *et al.*, 1972; Hootman and Conte, 1974; Kurata and Shigueno, 1976; Mykles, 1979). From the distribution of alkaline phosphatase in *P. setiferus*, it appears that the entire MGT is absorptive in the early larval stages. However, this apparent absorption is lost from the abdominal MGT during postlarval development. Other investigators also have concluded that the MGT in adults of *Penaeus* and other decapod crustaceans probably play a minimal role in absorption of organic nutrients, because only low rates of transport for amino acids, sugars, and vitamins could be measured across the MGT epithelium from the lumen (Ahearn, 1982, and citations therein; Chu, 1986). Even though small amounts of these solutes are transported from the lumen of the MGT into the cytoplasm of epithelial cells, intracellular metabolism of the solutes results in no net transepithelial flux into the hemolymph. From studies of carrier-mediated transmembrane transport systems for amino acids and glucose, it is also evident that relative rate of transepithelial solute transport in the adult decapod MGT by these carrier systems is almost two orders of magnitude lower than in the decapod hepatopancreas (Ahearn *et al.*, 1983, 1985, 1986; Ahearn and Clay, 1987a, b, 1988). These observations independently support the conclusion that the adult hepatopancreas is the primary area of absorption and that the MGT does not function significantly in this role. From histological and ultrastructural evidence, together with demonstration of *in vivo* uptake of radiolabeled solutes, it is usually inferred that the hepatopancreas is the primary site of nutrient absorption in adult crustaceans (Yonge, 1924; van Weel, 1955, 1970; Vonk, 1960; Speck and Urich, 1970; Dall, 1981).

The MGT in *Penaeus* is reported to function in ion transport and regulation of water flux from the midgut lumen to the hemolymph (Dall, 1967b; Talbot *et al.*, 1972; Ahearn *et al.*, 1978; Ahearn, 1982). Evidence for such a function is not surprising given the degree to which anal drinking and antiperistaltic water movements occur in some decapods (Fox, 1952; Pillai, 1960; Dall, 1965; Lovett and Felder, 1990a), and osmoregulatory function may, in part, account for observed ultrastructure of the MGT. Absence of alkaline phosphatase activity from the MGT is also consistent with an osmoregulatory function as alkaline phosphatase activity was not detected in either the gills or branchiostegites (primary osmoregulatory tissues) of *P. setiferus*.

Ontogeny of midgut function

Because the developing midgut tissue in embryos and nauplii of *Penaeus* functions in digestion and absorption of yolk, it is not unexpected that the entire midgut might retain similar functions during larval development. Even so, these functions are gradually lost from the abdominal MGT and from the anterior midgut caecum during larval and postlarval development. By the juvenile stage, only the hepatopancreas and that portion of the MGT within the cephalothorax are absorptive, while only the hepatopancreas functions in digestion. A similar ontogenetic change (from an undifferentiated and unspecialized larval gut to an adult gut in which functions are segregated) is also seen in teleost fish (Prakash, 1961; Blaxter, 1969) and may represent a general developmental phenomenon.

Dendrobranchiate shrimp such as *P. setiferus* may be unique among decapod crustaceans in their retention of both digestion and absorption in the anterior midgut caeca throughout larval development. In *Homarus*, after yolk material has been depleted during the first larval stage, the anterior caeca rapidly decrease in size. Similar to the change in the cells of the anterior caeca of *P. setiferus* after metamorphosis, there is a change in the epithelia of the anterior caeca in *Homarus* after the first larval stage: cuboidal, highly vacuolated cells are replaced by the highly columnar cells characteristic of the adult epithelium (Hinton and Corey, 1979).

Absorption in the abdominal MGT of larvae and early postlarvae may compensate for the small surface area in the anterior and lateral midgut caeca. In addition, because the gastric mill of the foregut is not functional during larval development, and because food has a relatively short retention time in the gut of larvae, retention of absorptive capacity along the entire length of the MGT could maximize assimilation of ingested food. As the simple lobes of the lateral midgut caeca ramify into the many tubules of the hepatopancreas, the relative surface area of this region of the gut increases substantially (Lovett and Felder, 1989). Thus, with loss of absorption in the anterior midgut caeca and the abdominal MGT, there is an increase in relative surface area (and hence absorptive capacity) of the hepatopancreas.

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